gPS Navigates Germ Cells to Pluripotency

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DOI 10.1016/j.stem.2009.06.012

The establishment of pluripotent stem cell lines from explanted testes has been hampered by a poor understanding of their cellular origin. In this issue of *Cell Stem Cell*, Ko et al. (2009) reproducibly generate pluripotent cell lines from murine testes and unequivocally demonstrate their origin from spermatogonial stem cells.

Pluripotent cell lines can be established from different sources, including embryonic stem cells (ESCs) from blastocyst-stage embryos, embryonic germ cells (EGCs) from primordial germ cells (PGCs), and induced pluripotent stem cells (iPSCs) from somatic cells upon overexpression of defined transcription factors (Hochedlinger and Plath, 2009). In addition, several groups have succeeded in deriving ESC-like cells from explanted murine and human testis cells (Conrad et al., 2008; Guan et al., 2006; Kanatsu-Shinohara et al., 2004, 2008; Kossack et al., 2008; Seandel et al., 2007). However, due to the low derivation efficiency of ESC-like cells from testes, and the lack of robust derivation protocols, the precise cellular origin of testis-derived pluripotent cells remained elusive. A functional and molecular comparison of the reported cell lines could not conclusively ascertain whether they originated from rare, residual PGCs, or rather from more committed spermatogonial stem cells (SSCs). In this issue of *Cell Stem Cell*, Ko et al. (2009) use clonal analyses to demonstrate that germline-derived pluripotent stem (gPS) cells arise from unipotent SSCs. In addition, the authors define specific culture parameters to consistently induce the switch from unipotent to pluripotent cells.

Robust Derivation of Pluripotent Cells from Adult Testis

In their study, Ko et al. cultured single Oct4-expressing, c-kit-negative testis cells isolated from an Oct4-GFP reporter mouse and derived stable SSC lines at a frequency of ~0.6%. Importantly, the resulting, cultured SSCs restore spermatogenesis upon transplantation into the testes of infertile mice, confirming their germline properties. Furthermore, when seeded at low plating densities, SSC cultures gave rise to spontaneous gPS cell colonies at a reproducible frequency of ~0.01% within 4 weeks. gPS cells were able to generate low-degree chimeras capable of germline transmission (Table 1), thus fulfilling all the requirements for a bona fide pluripotent cell type.

Prior to the present report from Schöler and colleagues (Ko et al., 2009), the cellular origin of gPS cells remained controversial. Previously, Seandel et al. attempted to address this question by demonstrating that rare pluripotent cell clusters emerged spontaneously within long-term cultured (3 months) SSCs (Seandel et al., 2007). However, the presence of a rare contaminating pluripotent cell type could not be excluded in this setting. In a separate study, Kanatsu-Shinohara and colleagues derived a pluripotent line that carried an apparently identical transgenic integration pattern as a starting SSC line. However, the possibility of rare identical integration events in two unrelated cells could not be formally excluded. An important advance of the current study is the demonstration that SSC lines can be established from single Oct4+c-kit− GSCs, and that gPS cells can be clonally derived from SSC lines, thus providing unequivocal evidence that unipotent SSCs are the cells of origin for pluripotent cells (Ko et al., 2009).

Characteristics of Testis-Derived Pluripotent Cells

In their seminal report demonstrating the derivation of so-called multipotent germline stem cells (mGSCs) from neonatal testes, Kanatsu-Shinohara and colleagues used unfractionated testes cells and selected reprogrammed colonies solely on the basis of their ESC morphology (Kanatsu-Shinohara et al., 2004) (Table 1). mGSCs formed differentiated teratomas and supported the development of chimeric mice. However, this methodology did not allow the derivation of mGSCs from adult mice unless p53-deficient animals were used, which are more prone to develop teratocarcinoma.

Neonatally derived mGSCs exhibited a genomic imprint pattern reminiscent of PGCs with largely erased imprints and some male-specific (androgenetic) imprints. Because imprinted DNA methylation marks are thought to be established around birth, it is likely that mGSCs were derived from relatively primitive germ cells that had not yet fully established their androgenetic imprints. Alternatively, the derivation procedure for mGSCs itself could have resulted in the erasure of some of the imprints, as is often seen in cultured ESCs. The current report revisits this issue and finds that male-specific imprints are maintained following conversion into gPS cells, suggesting that gPS cells may originate from a different type of germ cell than mGSCs (Ko et al., 2009). Since unbalanced genomic imprinting can result in tumor formation in mice, caution is warranted in a potential therapeutic application of human germ-cell-derived pluripotent cells.

The observation that genomic imprinting appears conserved in adult germ-cell-derived pluripotent cells raises questions about the origin of the previously reported multipotent adult germline stem cells (maGSCs) derived from the testes of a Stra8-GFP reporter mouse (Guan et al., 2006) (Table 1). In contrast to adult SSCs and gPS cells, maGSCs

Table 1. Summary of Reports of Murine Testis-Derived Pluripotent Cell Lines

	Kanatsu-Shinohara et al., 2004	Seandel et al., 2007	Guan et al., 2006	Ko et al., 2009
Donor cells	spermatogonial cell line established from total testis	GPR125-lacZ+ spermatogonial cell line	Stra-GFP+ spermatogonial cells	Oct4-GFP+ spermatogonial cell line
Age of donor mouse	neonates or p53-deficient adult mice	up to 1 year of age	4–6 weeks	5–8 weeks
Strain of mice	ddY and DBA/2	various strains	129/Ola, C57Bl/6, FVB	129Sv, C57Bl/6, FVB
Pluripotent cell line	mGSCs	MASCs	maGSCs	gPS cells
Conversion frequency	1 in 10^7 testis cells (0.00001%)	ND	4 lines from 15 mice	0.01%
Chimeras (stage)	adult	midgestation	adult	adult
Germline transmission	yes (by ICSI)	ND	yes (by PCR and lacZ)	yes (by PCR)
Imprint status	erased imprints with partially re-established androgenetic pattern	ND	somatic	androgenetic
Gene expression pattern	epiblast-like	epiblast-like	ESC-like?	ESC-like

ND, not determined; ICSI, intracytoplasmic sperm injection.

displayed a somatic imprinting pattern. Another difference compared with the other studies is the observation that Stra8-GFP mGSCs have the ability to both restore spermatogenesis upon transplantation into testes and form teratomas and chimeras upon injection into immunocompromised mice and blastocysts, respectively. This is a puzzling result, as testicular engraftment and teratoma/chimera-forming potential are usually mutually exclusive properties. Clearly, further work is needed to resolve the apparent discrepancies between the various studies.

Seandel and coworkers used an alternative spermatogonial marker, GPR125, to identify a population of germ cells that can give rise to multipotent adult spermatogonial-derived stem cells (MASCs) (Seandel et al., 2007) (Table 1). MASCs exhibited multilineage differentiation potential in vitro and in the context of teratomas and generated low-degree fetal chimeras in vivo. Interestingly, MASCs appeared to share several features with the recently reported epiblast stem cells (EpiSCs), which are pluripotent stem cells derived from murine postimplantation embryos (Lovell-Badge, 2007). These features include colony morphology and an epiblast-like gene expression pattern that excludes several ESC markers. Like MASCs, EpiSCs can generate teratomas and show negligible chimera contribution, demonstrating that these two cell types share both molecular and functional characteristics, suggestive of a similar pluripotent state. Notably, Kanatsu-Shinohara et al. also commented on the epiblast-like morphology of emerging mGSC colonies, and the gene expression data of mGSCs seem to support an epiblast-like signature of these cells (Kanatsu-Shinohara et al., 2004, 2008).

Different Pluripotent States of Testis-Derived Stem Cells?

In summary, several groups have reported the derivation of pluripotent stem cells from murine and human testes, which display different epigenetic, molecular, and functional properties. Murine pluripotent stem cells can exist in a variety of distinct pluripotent states depending on the stage of donor embryo, strain background, and derivation conditions. While the molecular and functional properties of previously described testis-derived stem cell lines appear consistent with an EpiSC-like pluripotent state, the gPS cells reported here by Schöler and colleagues display morphological and molecular characteristics akin to ESCs, including their ability to generate germline chimeras. It remains to be seen, however, whether these differences reflect different pluripotent states or result from different culture conditions, strain backgrounds, or reporter alleles, which are known to influence the pluripotent state of murine ESCs (Chou et al., 2008; Hanna et al., 2009; Hochedlinger and Plath, 2009). For example, the germ cell markers *Stra8* and *GPR125* may mark more differentiated subpopulations of spermatogonia compared with *Oct4*, which may identify more primitive cells. The advances reported by Ko and colleagues greatly facilitate further exploration of the influence of genetic background and microenvironment on the frequency and dynamics of converting restricted cells to pluripotency.

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